Iotonchium ungulatum n. sp. (Nematoda: Iotonchiidae) from the Oyster Mushroom in Japan¹

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Mycetophagous females, males and infective females of the genus *Iotonchium* which had not been known in Japan were found from gill-galled oyster mushrooms, *Pleurotus ostreatus*, which were collected at some localities of Tottori Prefecture in the late autumn of 1992 and 1993. By comparison with seven previously described species of *Iotonchium*, this nematode was named *I. ungulatum* n. sp. ("hiratake-hidakobu-senchu" in Japanese) and described here. The new species is apparently distinguished from *I. bifurcatum*, *I. californicum*, *I. cephalostricum*, *I. fungorum*, *I. imperfectum* and *İ. macrospiculatum* by having peculiar L-shaped spicules with a paw-like tip in the male (specific name is derived from this characteristic). From *I. mycophilum* whose male has not been known, the new species is differentiated by the shorter body length and the pointed tail terminus in the infective female. *Jpn. J. Nematol*. 31 (1/2):1-11 (2001).

Key words: gill-galled, L-shaped spicule, mycetophagous female, paw-like tip, *Pleurotus ostreatus*.

Some log-cultivated oyster mushrooms collected at some places in Tottori Prefecture in late autumn, 1992 and 1993, were sent to the author from a researcher in the Tottori Mycological Institute for the identification of inhabiting nematode. The oyster mushrooms showed the symptom of so-called gill-gall (1, 10), wart (9), white-gall (6) or gill-knot (13) disease (Fig.1 [A, B]).

A large female nematode with its spear and the eggs deposited were seen in each gall on the gill of the fruiting body at first. The eggs developed and grew up to be males and another type of females which were differently shaped from their mother nematodes in a few weeks. The males appeared first and the females were overwhelmingly dominant due to the early death of males at the time of decaying of the fruiting body.

The male and female nematodes grown from the eggs in the galls did not have valvular median esophageal bulb, and were sexually dimorphic, that is, the males had bilaterally symmetrical tri-lobed heads, while the females had symmetrical dome-shaped heads. Other characters of the nematode were a degenerate spear and L-shaped large spicules in the male and a female gonad consisting of rudimental ovary, short oviduct, long sperm-packed uterus and tubular vagina. Above-mentioned characters revealed that the nematodes were the males and infective females of a species of *Iotonchium* COBB, 1920 (4, 12), and the author named it *Iotonchium ungulatum* n. sp. ("hiratake-hidakobu-senchu" in Japanese as the causal nematode of the gill-gall disease of the oyster mushroom) by comparison with seven previously described species of *Iotonchium*. (The large nematodes in the gill galls of the fruiting bodies were

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the mycetophagous females [12] of this new species.) This is the first finding of the genus *Iotonchium* in Japan.

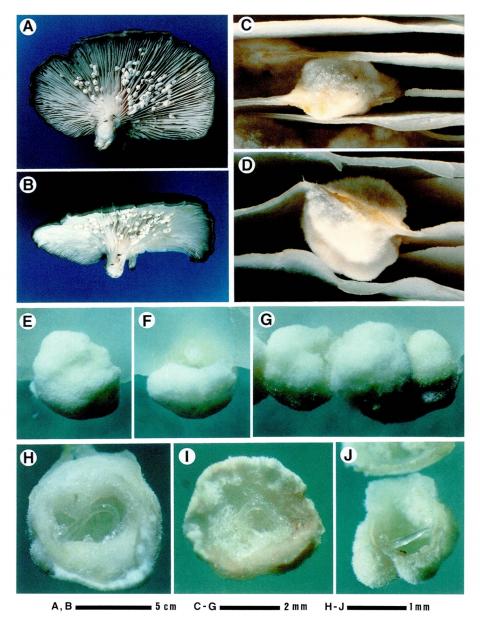


Fig. 1. Gill gall of oyster mushroom caused by mycetophagous female of *Iotonchium ungulatum* n. sp. A, B) Entire views of gill-galled oyster mushroom. A, backside view. B, lateral view. C, D) Horizontal views of gill gall. E-G) Lateral views of gill gall. E, side view of one gall. F, opposite side view of "E". G, cluster of three galls. H-J) Longitudinal sections of gill gall (one mycetophagous female and numerous deposited eggs are seen inside the gall, respectively).

MATERIALS AND METHODS

Specimens used in this report were obtained from the fruiting bodies of the oyster mushroom, *Pleurotus ostreatus* (JACQ.: FR.) KUMMER, collected at some localities in Tottori Prefecture and sent to the author (precise data are shown later).

Mycetophagous females and deposited eggs were obtained from the inside of fresh gill galls of the oyster mushroom by splitting the galls and by picking up the nematodes under a dissecting microscope. Males and infective females were separated from rotting fruiting bodies by the BAERMANN funnel technique. The nematodes were killed in hot water $(60-65^{\circ}C)$, fixed in TAF, and processed to glycerol.

Observations and drawings of the nematodes were done at magnifications of ca. $30\text{-}2,300\times$ under a microscope equipped with the differential interference contrast system of Nomarsky. Some spicules were obtained by soaking the males in 50% lactic acid and by dissecting them, and were also observed and drawn.

IOTONCHIUM UNGULATUM N. SP.

Measurements of mycetophagous females and deposited eggs (paratypes), males (holotype and paratypes), and infective females (allotype and paratypes) are shown in Tables 1-3, respectively.

Mycetophagous females and deposited eggs (Figs.1 [C-J] and 2). One female and numerous (ca. 500) deposited eggs, in general, found inside a gall on gill of fruiting body of oyster mushroom. Outside of gall whitish, velvety, ruggedly spheroid (ca. 2-3 mm in diameter). Inside of gall hollow (ca. 1-1.5

Table 1. Measurements of mycetophagous females and deposited eggs of *Ioton-chium ungulatum* n. sp.

Characters	Paratypes (n=20)
Mycetophagous females	
L (mm)	$2.55 \pm 0.41 (1.63 - 3.33)$
a	$23.4 \pm 1.9 \ (21.1 - 26.1)$
b	$18.5 \pm 2.9 \ (14.8 - 24.5)$
b´	? *
c	99.6 ± 21.0 (48.2 -126.4)
c´	$1.3 \pm 0.2 (0.8 - 2.0)$
V (%)	$97.8 \pm 0.5 \ (96.3 - 98.4)$
$\mathbf{G}_{1}\left(\% ight)$	$96.0 \pm 0.9 \ (94.0 - 96.9)$
Spear length (µm)	$8.3 \pm 0.3 (7.7 - 8.8)$
Distance from vulva to anus (μm)	$40.3 \pm 4.8 \ (29.7 - 47.7)$
Tail tip	
Length (µm)	$20.4 \pm 3.0 \ (13.1 - 24.9)$
Width (μm)	$5.4 \pm 0.7 (4.1 - 6.9)$
Length/Width	$3.8 \pm 0.8 (2.4 - 6.0)$
Deposited eggs	
Length (µm)	$100.2 \pm 5.8 \ (91.2 -108.5)$
Width (μm)	$53.7 \pm 2.5 (50.5 - 57.4)$
Length/Width	1.87± 0.12 (1.66- 2.08)

^{*}Posterior end of esophageal glands is unrecognizable.

Table 2. Measurements of males of Iotonchium ungulatum n. sp.

Characters	Holotype	Paratypes (n=20)
L (mm)	1.15	1.13± 0.05 (1.03- 1.27)
a	30.9	$31.3 \pm 2.9 \ (26.8 - 38.2)$
b	8.6	$8.4 \pm 0.4 (7.7 - 9.0)$
b´	8.2	$7.9 \pm 0.4 \ (7.2 - 8.4)$
c	20.3	$20.2 \pm 1.0 \ (17.7 - 21.8)$
c ´	2.7	$2.7 \pm 0.2 (2.4 - 2.9)$
T (%)	83.2	$83.3 \pm 0.9 (81.5 - 85.0)$
Position of deirids (%) *, **	11.3	$11.5 \pm 0.4 \ (10.3 - 12.5)$
Position of excretory pore (%) *,**	11.1	$11.9 \pm 0.5 \ (10.7 - 12.7)$
Position of nerve ring (%) *, * *	9.6	$9.6 \pm 0.5 (8.9 - 10.5)$
Spear length (µm)	4.7	$4.5 \pm 0.4 (3.9 - 5.2) ***$
Bursa length (µm)	102.3	$97.8 \pm 7.8 (82.3 -110.6)$
Spicules		
Proximal part length (µm)	26.5	$25.4 \pm 0.9 \ (23.8 - 27.1)$
Proximal part width (μm)	5.5	$5.1 \pm 0.3 (4.4 - 5.5)$
Distal part length (μm)	22.7	$21.8 \pm 0.7 \ (19.9 - 22.7)$
Distal pt.l./Proximal pt.l.	0.85	0.86± 0.04 (0.79- 0.93)

^{* (}Distance from head end to each part/L) \times 100.

mm in diameter), whitish, composed of rather rough and loose mycelia. Deposited eggs aggregated and adhering on internal surface of gall. Gall generated separately on gill, and sometimes in clusters.

Mycetophagous female body large (ca. twice as long as, and three times as wide as male or infective female), tapering at both ends, ventrally curved and more or less C-shaped after relaxation by gentle heat. Cuticle smooth with very fine transverse annulations. Lateral fields inconspicuous. Lip region offset, shaped like an inverted shallow dish without transverse stria. Cephalic framework divided into eight sectors. Spear short. Conical part of spear faintly recognizable (M: ca. 40%). Each basal knob of spear assymmetrical, extends laterally and slightly directed anteriorly. Anterior surface of basal knob slightly concave. Dorsal esophageal gland outlet opens in back of spear base. Esophagus short, usually pressed by overlapping ovary and obscure. Deirids, excretory pore, hemizonid and nerve ring unrecognizable. Ovary single, long, stretching forward to neck region and filling majority of body cavity (more than two thirds of body length). Posterior part of ovary usually sigmoid. Uterus usually packed with some eggs. Post uterine sac absent. Vulva a wide transverse slit, situated extremely posteriorly. Tail short and conoid, with a dorsally situated pointed tip shaped like a horn. Deposited egg ellipsoid. Ratio (length/ width) becomes low as egg develops.

Males (Fig. 3[A-M]). Body slender (but slightly shorter and thicker than infective famale), tapering at both ends, slightly arcuate ventrally after relaxation by gentle heat. Cuticle smooth with very fine transverse annulations. Lateral fields with two incisures (rarely, two more additional faint inner incisures present by thickening of original ones). Head bilaterally symmetrical, tri-lobed, flattened dorsoventrally. Spear degenerate, very short and slender, usually obscure. Basal knobs or thickenings of spear minute, roundish. Dorsal esophageal gland outlet inconspicuous. Deirids located at around level of excretory pore. Hemizonid situated just anterior to excretory pore. Nerve ring located anterior to level of

^{**} Position of central part.

^{***} n = 11.

Table 3. Measurements of infective females of *Iotonchium ungulatum* n. sp.

Characters	Allotype	Paratypes (n=20)
L (mm)	1.30	1.33± 0.06 (1.20- 1.45)
a	42.7	$45.4 \pm 2.1 \ (41.6 - 48.9)$
b	7.8	$7.6 \pm 0.6 \ (6.5 - 8.6)$
b´	6.1	$5.8 \pm 0.4 \ (5.3 - 6.5)$
c	12.6	$13.2 \pm 0.6 \ (12.2 - 14.4)$
c´	6.2	$6.0 \pm 0.4 \ (5.3 - 6.6)$
V (%)	85.4	$86.1 \pm 0.5 \ (85.0 - 87.1)$
G_1 (%)	38.4	$40.0 \pm 4.6 \ (33.0 - 50.7)$
Position of deirids (%) *,**	11.4	$11.0 \pm 0.5 \ (10.0 - 12.0)$
Position of excretory pore (%) *, * *	11.2	$11.1 \pm 0.5 \ (10.1 - 12.1)$
Position of nerve ring (%) *, * *	9.6	$9.3 \pm 0.4 \ (8.1 - 10.5)$
Spear length (µm)	14.9	$14.4 \pm 0.5 \ (13.5 - 15.2)$
Ovary length (µm)	56.7	$57.5 \pm 5.2 \ (46.3 - 68.4)$
Oviduct length (µm)	86.4	$46.9 \pm 38.8 (11.1 - 182.5)$
Uterus length (µm)	322.1	$400.5 \pm 68.2 (206.0 - 491.5)$
Vagina length (µm)	30.4	$25.5 \pm 6.2 \ (12.4 - 36.6)$

^{* (}Distance from head end to each part/L) \times 100.

excretory pore. Esophagus generally obscure. Esophageal glands overlapping intestine dorso-ventrally. Neither median esophageal bulb nor central valve apparatus present. Testis single, long, stretching forward almost to level of excretory pore, and filling majority of body cavity (more than 70% of body length). Sperms granular. Spicule large, shaped like obtuse-angled L, composed of proximal and distal parts. Head of spicule offset with slight constriction. Proximal part of spicule broad and slightly arcuate dorsally. Distal part of spicule extremely slender and strongly arcuate dorsally, with a paw-like tip which consists of bifid spatulate terminus and inward-directed bifid angular sub-terminus (specific name is derived from this characteristic). Anterior half of spicular distal part winged inward and connected to proximal part (wings of a pair of spicules overlap each other). Tip of spicular distal part generally protruded through cloaca after death. Gubernaculum absent. A pair of post-anal papillae present. Tail conoid, with a pointed terminus. Bursa large, completely enveloping tail.

Infective females (Fig.3 [N-U]). Body very slender (slightly longer and more slender than male), tapering at both ends, slightly arcuate ventrally after relaxation by gentle heat. Cuticle smooth with very fine transverse annulations. Lateral fields with two incisures (rarely, two more additional faint inner incisures present by thickening of original ones). Head offset, dome-shaped with very slight constriction. Cephalic framework divided into eight sectors. Spear rather long. Conical part of spear faintly recognizable (M: ca. 42%). Basal knobs or thickenings of spear triangular in outline, sloping posteriorly. Dorsal esophageal gland outlet opens in back of spear base. Deirids located at around level of excretory pore. Hemizonid situated just anterior to excretory pore. Nerve ring located anterior to level of excretory pore. Esophagus generally obscure. Esophageal glands overlapping intestine dorsally. Neither median esophageal bulb nor central valve apparatus present. Ovary single, short and rudimentary, consisting of ca. 10 cells. Oviduct slender, varying in length. Ovary and oviduct faintly recognizable. Uterus long (15-37% of body length), packed with sperms in the same shape and size as those in male. Vagina short,

^{**} Position of central part.

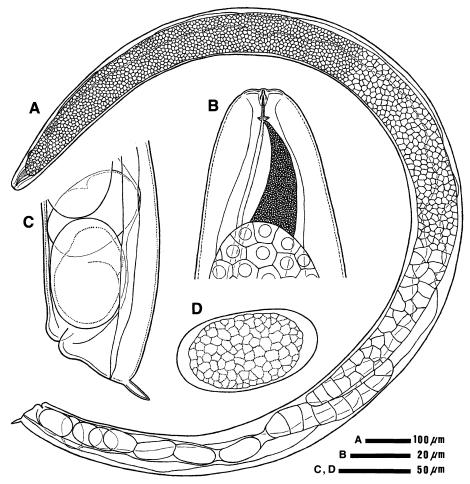


Fig. 2. Mycetophagous female and deposited egg of *Iotonchium ungulatum* n. sp. A-C) Mycetophagous female. A, entire body. B, head. C, posterior body. D) Deposited egg (entire).

tubular and bent. Post uterine sac absent. Vulva a narrow transverse slit. Flap-like cuticular appendage in back of vulval slit present (oval or elliptical in ventral view). Tail elongate-conoid, with a sharply pointed terminus.

Type host. Oyster mushroom, *Pleurotus ostreatus* (JACQ.:FR.) KUMMER: fresh fruiting bodies (mycetophagous females and deposited eggs found) and rotting fruiting bodies (males and infective females found).

Type locality. Okudani, Kooge Town, Yazu District, Tottori Prefecture, Japan.

Type specimens. Holotype (male) and allotype (infective female) obtained from the oyster mush-room collected in type locality on November 29, 1992 by Mr. Y.NOZAKI (slide nos.: 92IoM1 and 92IiF1, respectively). Paratypes (males, infective females, mycetophagous females and deposited eggs), the same data with holotype and allotype (slide nos.: 92IoM2-5, 92IiF2-5, 92ImF1-5 and 92IdE1-3, respectively). All the specimens are deposited at the Herbarium and Insect Museum of the National Institute of Agro-Environmental Sciences (NIAES), Kannondai, Tsukuba, Ibaraki, Japan.

Iotonchium ungulatum n. sp. was also found from the oyster mushrooms collected at the following

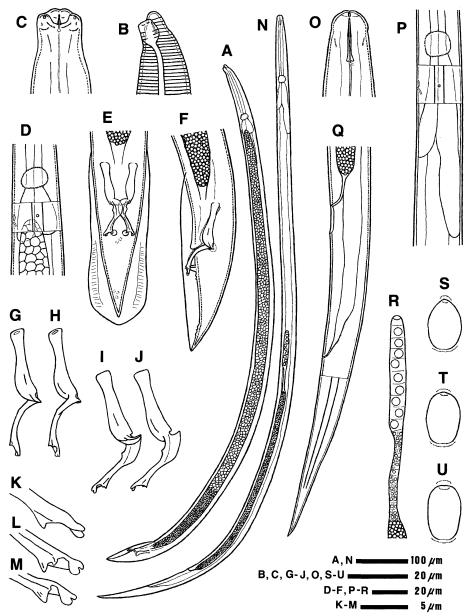


Fig. 3. Male and infective female of *Iotonchium ungulatum* n. sp. A-M) Male. A, entire body. B, C, heads (B, lateral; C, ventral). D, posterior esophageal region. E, F, posterior bodies (E, ventral; F, lateral). G-J, spicules (G, I, outside views of left spicule; H, J, inside views of right spicule; I, J, slightly pressed and distorted). K-M, spicule tips (K, outside; L,M, inside). N-U) Infective female. N, entire body. O, head. P, posterior esophageal region. Q, posterior body. R, ovary and oviduct. S-U, variations of flap-like cuticular appendage in back of vulval slit (ventral; S, oval; T, U, elliptical).

localities in Tottori Prefecture: Kokooge, Tottori City (col.: Nov. 30, '92 by Messrs. E. NAGASAWA & Y. MATSUDA); Shimosunami, Tottori City (col.: Nov. 1, '93 by Mr. T. HIASA); Ubeno, Iwakura, Tottori City (col.: Oct.26, '93 by Mr. M. IINO); and Yamada, Aoya Town, Kikoh District (col.: Nov. 12, '93 by Mr. Y. UEDA).

DIAGNOSIS

Iotonchium ungulatum n. sp. was compared with the following seven previously described species of *Iotonchium*:

- I. bifurcatum T. GOODEY, 1953 (8).
- I. californicum Poinar, 1991 (12).
- I. cephalostricum MEYL, 1954 (11).
- I. fungorum (Bütschli, 1873) Filipjev & Schuurmans Stekhoven, 1941 (2, 5).
- I. imperfectum (BÜTSCHLI, 1876) COBB, 1920 (3, 4).
- I. macrospiculatum (MEYL, 1954) J.B. GOODEY, 1956 (7, 11).
- I. mycophilum MEYL, 1954 (11).

The morphological characters compared in the males of the *Iotonchium* species are shown in Table 4 (*I. mycophilum* whose male has been unknown is excluded). *I. ungulatum* n. sp. may resemble *I. bifurcatum*, *I. californicum* and *I. fungorum* in having spicules shaped like obtuse-angled L with dorsally curved slender distal part (spicules of other species are shaped like right-angled or so L, with linear or ventrally curved distal part). However, the new species is apparently distinguished from these three species by its different ratio of spicule (distal part length divided by proximal part length: av. 0.86) and the peculiar paw-like tip of spicule (the ratios and the shapes of spicule tips are 0.33 and bifurcate in *I. bifurcatum*, 0.46 and teeth-like in *I. californicum*, and 1.00 and finger-like in *I. fungorum*). Other than the distinction by the spicule morphology, the new species is differentiated from (A) *I.cephalostricum*, (B) *I. fungorum*, and (C) *I. macrospiculatum* by the following characters, respectively: (A) longer body length, higher "a" and "c" values; (B) lower "a" value; and (C) higher "c" value.

The morphological characters of infective females of the *Iotonchium* species are compared in Table 5 (*I. imperfectum* can be omitted because its alleged female has an egg in the uterus [not having rudi-

Table 4. Males of *Iotonchium* spp.

species	L (mm)	a	c	Spicule		
				Shape*	D/P**	Tip
I. bifurcatum (8)	1.0-1.5	30-40	18-24	OL	0.33	Bifurcate
I. californicum (12)	0.8-1.3	33***	18***	OL	0.46	Teeth-like
I. cephalostricum (11)	0.5-0.7	19-23	8-11	RL	_	Rounded
I. fungorum (8)	0.9-2.2	40-50	15-22	OL	1.00	Finger-like
I. imperfectum (3)	?	?	?	RL	_	Blunt
I. macrospiculatum (11)	1.0-1.3	20-30	10-13	ML	_	Blunt?
I. mycophilum (11)		•••••	····· Male un	known ·····		•••••
I. ungulatum n. sp.	1.0-1.3	27-38	18-22	OL	0.86	Paw-like

^{*}ML (more or less L shape, with ventrally curved distal part),

OL (obtuse-angled L shape, with dorsally curved slender distal part),

RL (right-angled L shape, with linear distal part).

^{**}Distal part length / Proximal part length.

^{***} An approximate value calculated with the original measurements.

Table 5. Infective females of *Iotonchium* spp.

Species	L (mm)	a	С	V (%)	Spear (µm)	Tail terminus
I. bifurcatum (8)	1.2-1.5	50-60	14-15	81-84	20	pointed
I. californicum (12)	1.5-2.0	47-76	14*	85-87	18-21	pointed
I. cephalostricum (11)	1.0-1.1	33-43	13	88	23	blunt
I. fungorum (8)	2.8-3.7	75-93	12-14	81-84	15	pointed
I. imperfectum (3)	1.8**	23**	13**	88**	8**	pointed
I. macrospiculatum (11)	0.9-1.6	26-37	11-14	83-85	16.5***	blunt
I. mycophilum (11)	1.8-2.0	34-54	11-16	85-88	16-17	blunt
I. ungulatum n. sp.	1.2-1.5	42-49	12-14	85-87	14-15	pointed

^{*} An approximate value calculated with the original measurements.

mental ovary], and has not been regarded as the true female corresponding to the male [8]). *I. ungulatum* n. sp. strongly resembles *I. californicum* in body length, and "a", "c" and "V" values. However, the former can be distinguished from the latter by the shorter spear length. This new species can be differentiated from (A) *I. bifurcatum*, (B) *I. cephalostricum*, (C) *I. fungorum*, (D) *I. macrospiculatum* and (E) *I. mycophilum* by the following characters, respectively: (A) shorter spear length; (B) shorter spear length and pointed tail terminus; (C) shorter body length and lower "a" value; (D) higher "a" value and pointed tail terminus; and (E) shorter body length and pointed tail terminus.

Table 6 compares the morphological characters of mycetophagous females between *I. ungulatum* n. sp. and *I. californicum* (the only species of which female has been reported up to now). *I. ungulatum* n. sp. is distinguished from *I. californicum* by the longer body length, higher "c" and "V" values.

Table 6. Mycetophagous females of *Iotonchium* spp.

		^ ^			
Species	L (mm)	a	С	v (%)	Spear (µm)
I. californicum (12)	0.9-1.5	18-29	12*	84-88	9-11
I. ungulatum n. sp.	1.6-3.3	21-26	48-126	96-98	8- 9

^{*}An approximate value calculated with the original measurements.

REMARKS

Though GOODEY (8) presumed the presence of sexually-ripe gravid female living as a parasite of some fungus-inhabiting insect in 1953, the definition of the genus *Iotonchium* COBB, 1920, has long been based on the morphology of male and female with a rudimental ovary which were found from fungi. It was not until 1991 that three forms of an *Iotonchium* species (hexatyloid [mycetophagous female], *Iotonchium* [male and infective female with a rudimental ovary] and allantonematid [mature entomophagous female]) were found and described as *I. californicum* by POINAR (12).

The appearance of mycetophagous female of *I. ungulatum* n. sp. is considerably different from that of representative female of *Hexatylus*. It may be needed to study the taxonomic relationships between the mycetophagous females of *I. ungulatum* and the females of *Hexatylus*.

Larvae of a fungus gnat which was thought to be a host and a vector of I. ungulatum n. sp., were

^{**} Values of gravid female (not regarded as the true female corresponding to the male [8]).

^{***} A value cited from the redescription of J.B. GOODEY, 1956 (7).

also found boring into the fruiting body of the oyster mushroom. TSUDA et al. (13) verified the transmission of the nematode by a fungus gnat, Rhymosia domestica, and the gill-knot formation by the nematode. They already obtained some entomophagous females of the new species, and are going to describe the females soon (pers. comm.).

TSUDA et al. (13) called the "gall" on the gill "knot". Although the special calling , "knot", is adequate for the gall on the organ, such as root, which is shaped like a string, it may not be adequate for the gall on the gill which is shaped like a plate.

Among the Japanese names of the nematode-infected symptom of the oyster mushroom, "hidakobubyo (1, 10)", "ibo-byo (9)" and "shirakobu-byo (6)", hidakobu-byo ("gill-gall disease" in English) may be preferred, as it represents the symptom most pertinently.

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和文摘要

本邦産ヒラタケから検出した線虫の新種 Iotonchium ungulatum

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1992及び1993年の晩秋に鳥取県下の数地域で採集されたヒダに瘤のあるヒラタケから本邦未記録属線虫の1種の菌食態雌成虫、雄成虫及び感染態雌成虫を検出した。それらを既記載の7種と比較し、新種 Iotonchium ungulatum(ヒラタケヒダコブセンチュウ)と命名し、ここに記載した。本種の雄成虫の交接刺はL字形で、その先端部分が動物の脚に似た独特な形状をしていることから(種小名は、この特徴に由来する)、I. bifurcatum、I. californicum、I. cephalostricum、I. fungorum、I. imperfectum 及び I. macrospiculatum と明確に識別される。また、雄成虫が発見されていない I. mycophilum とは、本種の感染態雌成虫の体長が短く、尾の先端が尖っていることによって識別される。